Pristane Monitoring in Mussels

Project Number:	00195
Restoration Category:	Research and Monitoring
Proposer:	Jeffrey W. Short and Patricia M. Harris NMFS, Auke Bay Laboratory ABL Program Manager: Dr. Stan Rice NOAA Program Manager: Bruce Wright
Lead Trustee Agency:	NOAA
Cooperating Agencies:	None
Alaska Sea Life Center:	No
Duration:	Indefinite
Cost FY00:	$ 30,200
Cost FY01:	$ 30,000
Cost FY02:	$ 30,000
Cost FY03:	$ 30,000
Geographic Area:	Prince William Sound
Injured Resource/Service:	Pink Salmon, Pacific Herring

ABSTRACT

For the last 5 years, this project has focussed on elucidating the transport mechanism of pristane from *Neocalanus spp.* copepods into mussels during spring in Prince William Sound (PWS), and on monitoring the seasonal variation of pristane in these mussels. Results from these prior years indicate that the current network of stations sampled twice during May is sufficient to provide a 1-year advance indication of significant failure in the production of these copepods within PWS. Because these copepods are the key species linking primary productivity with higher trophic levels in PWS, a population failure would have serious ecosystem effects, including reduced catches of salmonids. Beginning this year the research component of this project will be dropped, and the sampling effort reduced considerably as guided by previous research. The objective of this monitoring effort is to provide advance warning of a "reverse regime shift" in PWS.

INTRODUCTION

This project has been funded for each of the last 5 years to investigate how pristane is transported from its source, *Neocalanus spp.* copepods, into mussels during spring in Prince William Sound (PWS). Previous work funded by this project has (1) elucidated the basic transport mechanism, which involves ingestion of *Neocalanus spp.* by juvenile fish followed by defecation of pristane - laden feces over mussel beds, (2) established that the seasonal timing of pristane incorporation into mussels always increases sharply in April and reaches a peak in May. and (3) shown that the network of 30 sampling stations monitored previously in PWS is probably necessary to cope with the high geographic variability found inter-annually. These results strongly suggest that monitoring this network of sampling stations twice during May, when pristane concentrations peak in mussels, could provide a reliable indicator of a *Neocalanus spp.* population crash, which would have serious ramifications for the rest of the PWS ecosystem, including commercial fisheries.

The carrying-capacity of Prince William Sound (PWS) for pink salmon depends crucially on the springtime abundance of large calanoid copepods, especially *Neocalanus spp*. The ultimate source of these oceanic copepods is the pelagic Gulf of Alaska, from which coastal populations are annually replenished. Prolonged interuption of this annual replenishment would almost certainly lead to a precipitous decline in the pink salmon carrying-capacity of PWS, and there is some evidence that such declines have occurred in the past. The pristane monitoring program proposed here would provide a 1-year advance warning should such a decline recur, at an annual cost of $30K/yr. This would provide time for economic adjustment by the commercial fishing industry and PWSAC hatcheries, and would alert the interested scientific community to an opportunity to study an impending ecosystem shift in real time.

Neocalanus spp. are the dominant zooplankters during spring in PWS because of their life-history pattern, the presence of suitable overwintering habitat in PWS, and the proximity of PWS to the GOA. Adult *Neocalanus plumchrus* reproduce in winter at depths below about 300 m, and developing copepodites migrate to the surface to graze the spring phytoplankton bloom. Their grazing is so efficient that they typically account for more than 90% of the zooplankton biomass in April and May, when juvenile pink salmon begin near-shore marine residence. The required deep-water winter habitat is present throughout much of the northwesterly part of PWS, but is elsewhere usually absent on the northern continental shelf of the GOA. Within PWS, developing copepodites suffer heavy predation, and survivors tend to be advected out of the Sound and then westward by the Alaska Coastal Current (ACC) before their migration to deeper water in mid-summer. The over-wintering populations of *Neocalanus spp*. in PWS are replenished from pelagic GOA populations through cross-shelf advection into the Sound from the east.

The ability of the GOA to supply *Neocalanus spp*. to coastal depressions such as in PWS has increased substantially over the past few decades. Zooplankton biomass about doubled in the GOA from the early 1960s to the early 1980s, which resulted in much higher abundances adjacent to the continental shelf of the northern GOA (Brodeur and Ware, 1992, Fish. Oceanog. **1**:32). This was attended by a concurrent increase in salmon catch rates. In PWS, pink salmon catches increased by a factor of 5 in 1979 compared with the resat of that decade, and chum salmon

showed a similar increase in 1980. Catches of both species have remained well above norms for the 1970s ever since. This suggests a dramatic improvement in survival of juveniles these two species in 1978 inside PWS, perhaps a consequence of the 1977 "regime shift" thought to have affected the atmospheric and ocean current circulation of the GOA. A shift in the opposite direction, leading to a contraction of *Neocalanus spp* abundances in the GOA and conequent decimation of near-shore populations such as within PWS, could be expected to result in a precipitous decline in juvenile salmonid survival within the Sound. Loss of the efficient phytoplankton grazer in early spring would result in much more of the primary productivity becoming shunted to the benthos, and unavailable to the developing salmonids.

The proposed project would assess large inter-annual changes in abundances of *Neocalanus spp* within PWS by monitoring concentrations of pristane in mussels during spring. Pristane is a hydrocarbon biosynthesized from chlorophyll by herbivorous copepods in the genera *Calanus* and *Neocalanus*. These copepods are the only proven modern marine source of pristane (Avigan & Blumer, 1968, J. Lipid Res. **9**:350;), and they typically contain concentrations that approach 1% dry weight (i.e. 10,000,000 ppb). As a branched alkane, pristane is highly lipophilic and resistant to metabolic degradation. Pristane concentrations that range to 70,000 ppb (dry weight) are found in filter feeding organisms such as mussels and some clams in PWS during spring. Research at the Auke Bay Laboratory (ABL) sponsored in part by this project in previous years has established that in PWS, pristane is incorporated into mussels through ingestion of fecal material produced by juvenile fish preying on *Neocalanus spp.* Requirements for high concentrations of pristane to appear in mussels include high abundances both of *Neocalanus spp.* and of juvenile fish predators within a few 10s of meters of mussel beds. Juvenile pink salmon are particularly effective at transferring pristane from *Neocalanus spp.* to mussels because they spend much of their early marine residence preying almost exclusively on these copepods and defecating directly above mussel beds. Other ABL experiments have confirmed that the presence of *Neocalanus spp.* alone is not sufficient to result in much transfer of pristane to mussels in PWS.

The above results confirm that analysis of pristane in mussels may be used to monitor the PWS marine ecosystem. Dramatic increases in pristane concentrations are a regular seasonal feature of PWS, having appeared in 13 of 14 years monitored during the last 22. The exception was 1977, the year monitoring began, and the ensuing year was the last year of depressed salmon catches in PWS. This is consistent with the scenario described above regarding the introduction of and dependence of pink salmon on *Neocalanus spp.* in PWS, and strongly suggests that failure to detect much pristane in mussels during spring would foreshadow a dramatic decline in salmonid survival to the following year. Poor survival would result from the absence of *Neocalanus spp.*, thereby un-coupling primary production from juvenile salmon in PWS and eroding the carrying-capacity of the Sound.

This proposal signals the transition of this project from a research and monitoring project to a purely monitoring one. This transition exploits the results of the research phase to permit an efficient monitoring design at minimum cost. Two samplings are proposed, one each in late April and late May, to address the temporal variability of the spring peak of pristane concentrations in mussels observed during prior years, and to verify the absence of pristane if observed during the first sampling. The current network of sampling stations is retained because geographic variability

is high, and most of the sampling costs are fixed, so drastic reductions in the number of sampling stations are necessary to achieve significant cost reductions.

NEED FOR THE PROJECT

A. Statement of Problem

Determination of the causes of the dramatic declines in populations of pink salmon, herring and fish-eating seabirds following the *Exxon Valdez* oil spill requires an assessment of the natural factors that affect recruitment and survival of these species, because any negative effects of the spill may be confounded by these natural factors. In addition, natural factors impose constraints on the recovery potential of these species. Pink salmon have been identified as recovering; herring, pigeon guillemots, cormorants, and marbled murrelets are identified as not recovered. If population declines of these species are the result of changes in the basic ecology of Prince William Sound due to natural phenomena (e.g. El Nino), then recovery of these populations to pre-spill levels may not be possible, and the criteria for recovery must recognize these changes.

B. Rationale

The proposed project will continue to provide information that may be used to evaluate the effect of natural constraints on the recovery of Prince William Sound pink salmon and herring populations and secondarily, on fish-eating marine birds. Annual monitoring of pristane concentrations in mussels will permit an indirect evaluation of the effects of juvenile survival on recruitment.

It is proposed that this basic monitoring be supported indefinitely as a forecasting "insurance policy" for PWS fisheries in the event of a reversion to ecosystem conditions typical of the late 1960's and early 1970's, when the salmon recruitment was much lower than at present. Such conditions could recur if *Calanus* and *Neocalanus spp.* populations in the GOA contract in response to a regime shift in oceanic currents, and thereby fail to re-populate the marine depression system of northwestern PWS. Absence of these over-wintering zooplankters in PWS would substantially un-couple primary from secondary production the following spring, resulting in a sharply reduced forage base for juvenile salmonids and other zooplanktivorous fishes. This could lead to a corresponding reduction in recruitment for the affected species. The ability to forecast such events could substantially ameliorate the consequent adverse economic impacts.

C. Location

Mussel samples will be collected in Prince William Sound and will be analyzed for pristane concentrations at the Auke Bay Laboratory, Juneau, Alaska. The identification of important productive areas in PWS and inter-annual productivity data will be useful to local fishery and hatchery managers. Educational materials and the brochure will be most appropriate for residents and students of Prince William Sound, but will also be available for others.

COMMUNITY INVOLVEMENT

We will continue to involve Prince William Sound residents in this project to share knowledge and interest in PWS ecosystems and to reduce sampling costs. Since 1994, the Prince William Sound Aquaculture Association has collected mussels near their 4 hatcheries at the appropriate times and stored them until the end of the season for pick-up. This year students with Youth Area Watch (Project 99210) and independent students will again be collecting mussels near their hometowns, Tatitlek, Whittier, Chenega, Kenny Cove, Valdez, Cordova, and Seward, and may be assisting with collections at other sites. We will provide materials for each participating school that explains the rationale of the project, and compares specific results for each school with the results for the whole effort. The underlying biology of this project gets to the heart of how the sound turns sunlight into fish, which we believe can provide a very useful local teaching resource. Youth Area Watch students will also continue to participate in a 1 day workshop at Auke Bay Laboratory on laboratory analysis techniques for pristane in mussels.

PROJECT DESIGN

A. Objectives

In 2000 and onward this project has 1 objective:

1. Measure pristane concentrations in mussels collected during late April and again in late May from 30 stations in Prince William Sound to evaluate inter-annual variability.

B. Methods

The project objective will be addressed by determining the variability of pristane concentrations in mussels (*Mytilus trossulus*) from 30 sites in PWS during late April and again in late May. Collected mussels will be stored frozen and analyzed for whole-body pristane concentration. Mussels (20) will be collected from selected mussel beds and placed into a plastic bag together with collection documentation (i.e. date, time, location, collector). Selected mussels will ideally be in the length range 20 - 45 mm. Mussels are collected along a transect parallel with the shoreline; 1 mussel is collected every consecutive meter. Previous results archived in the *Exxon Valdez* restoration database for hydrocarbons indicates that pristane concentrations in mussels collected in this way are representative of entire mussel beds.

Pristane concentrations in mussels will be compared with mean concentrations from corresponding stations collected during the period 1994 - 1999, when *Neocalanus spp.* was abundant during spring in PWS. Consistent failure to detect one-tenth of this mean at all stations and at both sampling times will be used as criteria for a significant (and serious) decline in in the *Neocalanus spp.* forage base. Statistical modelling studies conducted in prior years indicate that such a decline would be extremely significant in the statistical sense; the factor of 10 is used becuse declines of this magnitude (or larger) are suggested by the postulated replenishment-failure mechanism described above (see Introduction).

The chemical analysis of pristane involves pentane extraction of macerated tissues, lipid removal with silica gel, and separation and measurement of pristane by gas chromatography equipped with a flame ionization detector. Pristane measurement will use the internal standard method, with deuterated hexadecane and deuterated eicosane added to the pentane initially as the internal standard. Pristane identification will be based on retention time relative to the internal standard. Quality control samples include method blanks, spiked method blanks, and reference sample analyzed with each batch of 20 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Recovery of the internal standard will be determine by adding a second internal standard prior to instrumental analysis. Method detection limits will be assessed annually for the mussel tissue matrix, and these detection limits will be assumed for the other matrixes analyzed. Based on previous performance, we anticipate accuracy of " 15% of National Institute of Science and Technology (NIST)-certified values for the spiked blank and reference samples, precision of 95% of reference samples within " 15% of sample means, and laboratory artifacts below detection limits more than 99% of the time. This level of analytical performance will insure that variability due to sample analysis is negligible compared with variability among replicate mussel samples.

Percent moisture will also be determined in samples so that results may be analyzed on dry weight weight bases. Dry weights will be determined by heating samples at 60 C to constant final weight.

Because there is no other practical way of estimating energy conversion from *Neocalanus* to their near-shore predators over a broad geographic area such as PWS, there are no alternative methodologies to consider here.

C. Contracts and Other Agency Assistance

There will be no contracts under this project.

SCHEDULE

A. Measurable Project Tasks for FY99

FY00:

Apr 15 - May 30: Collect mussel samples.

Jun 1 - Sep 30: Analyze 1999 samples for pristane, summarize results in a report

B. Project Milestones and Endpoints

Write report by Sep. 30, 2000

C. Completion Date

Sep. 30, 2000

PUBLICATIONS AND REPORTS

An annual report will be produced by September 30, 2000.

NORMAL AGENCY MANAGEMENT

NOAA/NMFS has statutory stewardship for most living marine resources; however, if the oil spill had not occurred, NOAA would not be conducting this project. NOAA/NMFS proposes to make a significant contribution (as stated in the proposed budget) to the operation of this project, making it truly cooperative.

COORDINATION AND INTEGRATION OF RESTORATION EFFORT

We are cooperating closely with Youth Area Watch (99210), which is providing us with samples and to whom we are providing training and educational materials.

EXPLANATION OF CHANGES IN CONTINUING PROJECTS

The changes are all reductions in scope resulting from transition to a purely monitoring project.

PROPOSED PRINCIPAL INVESTIGATOR

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PRINCIPAL INVESTIGATOR

Jeffrey W. Short

Education:
BS, 1972, University of California, Riverside (Biochemistry & Philosophy)
MS, 1982, University of California, Santa Cruz (Physical Chemistry)

Relevant Experience:
1989- Present: Established and managed the hydrocarbon analysis facility at ABL to analyze hydrocarbon samples generated by the *Exxon Valdez* NRDA effort (about 20% of these samples were analyzed at ABL).
1989 - 1992: Principal Investigator, Exxon Valdez project Air/Water #3: Determination of petroleum hydrocarbons in seawater by direct chemical analysis and through the use of caged mussels deployed along the path of the oil spill.
1991 - 1996: Principal Investigator, Exxon Valdez project Subtidal #8: Development of computer-based statistical methods for global examination of sediment and mussel hydrocarbon data produced for the Exxon Valdez NRDA effort for systematic bias, and for identification of probable sources of hydrocarbons. In addition, this project produced both hard-copy and computer display maps of all the sediment and mussel hydrocarbon data.
1994 - 1995: Initiated data analysis and pilot projects that established the role of pristane in Prince William Sound.
1996-1997 Principal Investigator 96195 and 97195

OTHER KEY PERSONNEL

Patricia M. Harris

Education: University of Alaska Fairbanks; B.S. Biological Science 1966
Graduate work at U of A Fairbanks, U of A Southeast, University of British Columbia
Relevant Experience:
1989-1992: Co-principal investigator of NRDA study Subtidal 3, was responsible for field logistics and sample collection and assisted in data analysis and report preparation; also assisted other NRDA projects in field collections.
1992 -1996: participated in study design, field work, proposal preparation, data analysis, and report preparation for mussel bed monitoring and restoration (R103-96090).
1994-1997 Participated in logistic planning, sampling, and community involvement coordination for the pilot pristane project ,96195, and 97195.

Relevant publications: Co-author of final reports for NRDA study Subtidal 3 and several publications pertaining to distribution of *Exxon Valdez* oil in mussels and underlying sediments. Several public presentations of oil-related scientific research.

Responsibilities: Coordinate sample collection logistics and collect mussel samples; data analysis; report and proposal preparation; and preparation of science educational materials, posters, and

reports.